

BLOOD ANTIOXIDANT STATUS IN COCCIDIOSIS-INFECTED BROILER CHICKENS FED ALLIUM SPP.-BASED SUPPLEMENTS

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ABSTRACT

This study aimed to evaluate the dietary effect of dried onion (*Allium cepa*) and Garlicon, a product rich in propylpropanthiosulfinate (PTS) and propylpropanthio-sulfonate (PTSO), on antioxidant indicators in the blood plasma of broiler chickens challenged to *Eimeria* spp. infection. Chickens were divided into four dietary groups with specific treatment (CP, control positive group receiving a control diet with synthetic coccidiostat; CN, control negative group receiving a control diet without supplements; ON1, experimental group receiving a diet supplemented with 7,5 g/kg onion meal; ON2, experimental group receiving a diet supplemented with 30 mg/kg Garlicon). At 21 and 42 days of age, no statistically significant differences were found in catalase and glutathion-S-transferase activity, or malondialdehyde level, indicating no significant effect on lipid peroxidation in blood plasma. A significant increase in glutathione peroxidase (GPx) activity was observed at 21 days of age in the ON1 group (1329.97 ± 62.217 nmol NADPH/min/ml; $P < 0.01$), and in ON2 group (1225.12 ± 96.029 nmol NADPH/min/ml; $P < 0.05$) compared with the CP group (915.42 ± 86.754 nmol NADPH/min/ml). At 42 days, GPx activity remained significantly higher in the ON1 group (1485.07 ± 78.595 nmol NADPH/min/ml; $P < 0.05$) while a highly significant increase was also detected in the CN group (1681.29 ± 58.741 nmol NADPH/min/ml; $P < 0.01$) compared with the CP group (1120.80 ± 102.672 nmol NADPH/min/ml). The results suggest that supplementation with dried onion meal, and Garlicon did not reduce lipid peroxidation in plasma, but it positively affected antioxidant enzyme activity, especially GPx. Further studies dealing the effect of *Allium* spp.-based phyto-genic additives on chickens' health and metabolism are needed to evaluate with regards to growth rate dynamics.

Keywords: antioxidants; onion; broilers; glutathione peroxidase



INTRODUCTION

Oxidative stress affects the overall health of chickens in modern production systems. It arises from an imbalance between the formation of reactive oxygen species and the antioxidant defense mechanisms of the organism. In broiler chickens, it can lead to negative effects on growth, health status, immunity, and overall performance. Impaired antioxidant protection further increases their susceptibility to infections (Oke et al., 2024). A current trend in this area of research is the effort to identify suitable alternatives to synthetic additives that support improved performance and health status of broiler chickens, in order to reduce environmental impact caused by residues and to provide potential benefits for consumer health (Adeyemi et al., 2021; Šimek et al., 2026; Zapletal et al., 2026). In this regard, plants of the genus *Allium* are promising, as they are an important source of organosulfur compounds with proven antioxidant, antimicrobial, and immunomodulatory effects, and they are also a source of polyphenols, especially the flavonoid quercetin, a strong antioxidant that is present at higher levels in onion (*Allium cepa*) (Marefati et al., 2021; Cascajosa-Lira et al., 2024; Aditya et al., 2017). Phytochemical compounds derived from these plant species, particularly propyl thiosulfinates (PTS) and propyl thiosulfonates (PTSO), derivatives of propiin, also appear to be highly effective. These compounds are stable, which makes them suitable for use in feed, and they have been shown to possess marked antimicrobial activity (Aguinaga-Casañas et al., 2022; Cabello-Gómez et al., 2022; Cascajosa-Lira et al., 2024). The aim of this study was to compare the effects of dried onion, and Garlicon product, which contains high levels of PTS and PTSO, and to demonstrate their impact on reducing lipid peroxidation and on modulating the activity of antioxidant enzymes in blood plasma.

MATERIAL AND METHODS

The feeding trial (a total of 192 ROSS 308 hybrid chickens) was performed in the approved experimental facility of Department of Animal Breeding, Animal Nutrition and Biochemistry, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Czech Republic. Male chicks were divided into four dietary groups (CP, control positive group receiving a control diet with synthetic coccidiostat; CN, control negative group receiving a control diet without synthetic coccidiostat; ON1, experimental group receiving a diet supplemented with 7,5 g/kg onion meal; ON2, experimental group receiving a diet supplemented with 30 mg/kg Garlicon) were used in this study. Garlicon product was provided by DOMCA SAU (Granada, Spain). A two-phase feeding programme was used, with grower and finisher diets provided across all four dietary treatments. With respect to other health evaluation, all chickens were challenged to *Eimeria* spp. Chickens were housed in floor litter pens according to management handbook for ROSS 308 (Aviagen 2025).

At each sampling time (21 and 42 days of age), 32 male broiler chickens ($n = 8$ per dietary group) were randomly chosen for blood sampling. After recording the live weight of the birds, blood samples were obtained via puncture of the *v. basilica* using an aspiration syringe with sodium heparin as an anticoagulant. Each blood sample was centrifuged at 3000 rpm for 10 min at 20 °C to obtain plasma. Then samples were stored at -80 °C until further biochemical and oxidative stress analyses.

Plasma total protein (TP) was measured using an automated analyser (Indiko, ThermoFisher Scientific, USA) with commercially available kit (DiaSys Diagnostic Systems GmbH, Germany). The plasma sample was used to determine catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) activities. MDA, GST, and GPx were measured using a Varioskan Flash multimode reader (Thermo Fisher Scientific, Brno), while CAT activity was measured using a GENESYS 150 UV-visible spectrophotometer. The parameters were measured according to the methodology previously described in the study by Blahová et al. (2013).

Statistic analysis was performed using STATISTICA CZ version 10. After Shapiro-Willk W test, and K-S & Lilliefors test, one-way ANOVA was used. When ANOVA showed significant effect, Tukey *post-hoc* test was used. Significance was considered at $P < 0.05$ (*), and $P < 0.01$ (**).

RESULTS AND DISCUSSION

Tab. 1: Selected antioxidative blood plasma indicators in ROSS chickens with regard to dietary treatment.

Indicators	Dietary groups					P			
	CP		CN		ON1		ON2		
	x	SEM	x	SEM	x	SEM	x	SEM	
Age 21 days									
LW (g)	701.00 ± 19.168		726.50 ± 28.359		705.00 ± 11.220		733.38 ± 32.734		n.s.
TP (g/L)	20.14 ± 0.680		21.80 ± 1.079		23.54 ± 0.780		23.03 ± 2.661		n.s.
CAT (μmol/min/mL)	15.73 ± 0.764		15.16 ± 2.351		12.32 ± 0.785		13.69 ± 2.012		n.s.
MDA (μmol/L)	0.35 ± 0.046		0.33 ± 0.031		0.37 ± 0.020		0.33 ± 0.052		n.s.
GST (μmol/min/L)	23.34 ± 1.867		24.55 ± 1.604		29.04 ± 1.248		23.38 ± 2.221		n.s.
GPx (nmol NADPH/min/mL)	915.42 ^{A,a} ± 86.754		1122.76 ± 40.905		1329.97 ^B ± 62.217		1225.12 ^b ± 96.029		**
Age 42 days									
LW (g)	2454.75 ^a ± 47.298		2743.25 ^b ± 94.166		2476.00 ^a ± 23.337		2579.75 ± 39.964		*
TP (g/L)	25.21 ± 4.345		27.22 ± 1.863		25.60 ± 2.797		25.77 ± 3.687		n.s.
CAT (μmol/min/mL)	78.94 ± 2.263		79.56 ± 2.251		87.54 ± 2.356		85.75 ± 3.128		n.s.
MDA (μmol/L)	0.26 ± 0.014		0.24 ± 0.034		0.26 ± 0.031		0.24 ± 0.021		n.s.
GST (μmol/min/L)	30.90 ± 2.490		32.89 ± 1.048		29.40 ± 2.046		34.66 ± 1.740		n.s.
GPx (nmol NADPH/min/mL)	1120.80 ^{A,a} ± 102.672		1681.29 ^B ± 58.741		1485.07 ^b ± 78.595		1336.10 ± 107.325		**

CP, control positive; CN, control negative; ON1, experimental group fed onion meal supplementation; ON2, experimental group fed Garlicon supplementation; LW, live weight; TP, total protein; CAT, catalase; MDA, malondialdehyde; GST, glutathion-S-transferase; GPx, glutathion peroxidase; ns, not significant ($P > 0.05$); *, means within row differs significantly at level $P < 0.05$; **, means within row differs significantly at level $P < 0.01$.

As shown in Table 1, no statistically significant effects were observed at either 21 or 42 days of age on CAT and GST activity or on MDA levels as an indicator of ongoing lipid peroxidation. Total plasma protein was also not significantly affected, and the values remained within the physiological range for broiler chickens reported by Zálešáková et al. (2025). Besides that, in 21-day-old chickens, the chosen dietary phytogenic additives did not significantly affect body weight ($P > 0.05$). A statistically highly significant increase in GPx activity, an important antioxidant enzyme, was found in group ON1 as compared to CP group (+414.55 nmol NADPH/min/ml, $P < 0.01$), and compared to ON2, (+309.70 NADPH/min/ml, $P < 0.05$). In 42-day-old chickens, we also found a highly significant increase in GPx activity

in group CN with comparison to CP (+560.49 nmol NADPH/min/ml, $P < 0.01$). Concerning the experimental groups, chickens receiving a diet supplemented with onion meal at rate of 7.5 g/kg of diet (group ON1) reached significantly higher GPx activity as compared to control positive group (+364.27 nmol NADPH/min/ml, $P < 0.05$). The highest final LW was found in chickens of CN groups as compared to CP and ON1 (+288.50 g and +267.25 g, resp., $P < 0.05$) groups. In neither sampling period, similarly to the study by Vossen et al. (2011), were we able to demonstrate the expected effect of the selected phytogetic additives on reducing lipid peroxidation; instead, only the effect of dried onion and Garlicon on the antioxidant enzyme GPx was observed. Our findings are consistent with study of Adeyemi et al. (2021), who reported increased antioxidant enzyme activity in blood plasma and tissues in broiler ROSS 308 chickens supplemented with onion skin waste. The antioxidant effect of PTSO in chickens has not been sufficiently studied, although its antimicrobial effect has been demonstrated to a greater extent (Peinado et al., 2012; Aguinaga-Casañas et al., 2022; Cabello-Gómez et al., 2022) but our findings demonstrate its effect on increasing antioxidant enzyme activity, especially in younger 21-day old chickens. Aditya et al. (2017), in a study in which they supplemented broiler chickens with onion extract, showed that dietary supplementation affected lipid peroxidation in breast muscle but they did not assess antioxidant indicators in blood plasma. The effect of feed supplementation with onion and garlic, as principal representatives of the genus *Allium*, on antioxidant enzymes was also demonstrated by Sallam et al. (2024), who added onion and garlic juice to the drinking water of broiler chickens; in their study, a statistically significant increase in CAT, GST, and superoxide dismutase was observed in homogenized liver tissue, where the activity of these enzymes is higher than in blood plasma.

CONCLUSION

Supplementation of broiler chicken feed mixtures with dried onion meal, and Garlicon did not reduce lipid peroxidation in blood plasma of chickens challenged with *Eimeria* spp. The main finding of the study was GPx increase in with the strongest effect observed for dried onion. Further studies dealing the effect of *Allium* spp.-based phytogetic additives on chickens' health and metabolism are needed to evaluate with regards to growth rate.

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